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REFERENCES

- BACKHOUSE, T. C. (1939) *Lancet*, ii, 736.
O'MEARA, P. J. (1940) *J. Roy. Naval Medical Service*, **26**, 284.
PLUMMER, N., and McLELLAN, F. (1940) *J.A.M.A.*, **114**, 943.
SMITH, E., EVELYN, K. A., and NOLAN, J. F. (1940) *J. Canadian Med. Ass.*, **42**, 27.
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III

SOME FACTORS AFFECTING THE DEVELOPMENT OF IMMUNITY IN EXPERIMENTAL RABBIT-SYPHILIS

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THE object of this paper is to bring to notice some hitherto unpublished experiments to see if simultaneous inoculation of a rabbit with a few strains of *S. pallida* and the consequent provocation of a large reaction in the first instance would make the animal resistant to superinoculation with other strains, in other words, develop a pan-immunity as contrasted with a mono-immunity. Before, however, dealing with these experiments, which I carried out in 1930-32 in the Bacteriological Department of the Reichsgesundheitsamt, Berlin-Dahlem, it seems appropriate to summarize existing knowledge on some factors affecting the acquisition of immunity in experimental syphilis of the rabbit.

In the investigation of experimental rabbit-syphilis two questions are of outstanding interest. (a) Is there any "true" immunity? and (b) are the immunological conditions in the syphilitic rabbit essentially different from those in the syphilitic patient? As regards the first question, the majority of investigators [Chesney and Kemp (1924), Chesney (1926, 1930), Manteufel and Worms (1927), Uhlenhuth and Grossmann (1927, 1928), Breinl and Wagner (1929), Manteufel and Herzberg (1933), Breinl (1935), Tani and Aikawa (1936), (1940), Vászárhelyi (1936)] hold that the syphilitic rabbit acquires a "true" immunity, that is one which does not necessarily depend on the persistence of *S. pallida* in the body, but remains after disappearance of this organism. Whether the syphilitic rabbit acquires a pan-immunity such as is said to

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exist in human syphilis or merely a mono-immunity against the strain with which it was infected is a question which does not appear to me to be settled, although most authors seem to have answered it in the affirmative.

In order to obtain a proper idea of the immunological conditions prevailing in the syphilitic rabbit it seems advisable to consider only experimental conditions which do not admit of ambiguous interpretations. For these reasons only superinfections, and not reinfections, will be considered here, that is to say the results of reinoculations in *untreated* rabbits. For the same reason ocular inoculations will not be discussed as the eye plays a special part in the immunity of the syphilitic rabbit.

The factors which will be discussed here in their relation to the result of superinoculation in untreated syphilitic rabbits are (1) the interval between the first and second inoculation; (2) the quality (the virulence) and the amounts of inocula used in the first and the second inoculation; (3) the methods and the sites of the first and the superinoculations; and (4) the reaction of the rabbit to the first inoculation.

(I) THE TIME-FACTOR

Here I shall deal only with homologous superinoculations; the heterologous ones will be considered in the next section. Further in what follows the words "first infection," unless otherwise stated, means development of local and/or general syphilitic affections, and a superinoculation is not called positive or successful here unless syphilitic manifestations develop as a result of it.

It has been shown by numbers of workers, notably Tomaszewski (1910) and Kolle (1922 and 1924), that in general the success of superinoculation of an untreated syphilitic rabbit depends on *inter alia* the time interval between the first and the second inoculation carried out by the scrotal or testicular route. In agreement with Kolle (1922 and 1924), they found that superinoculation within 40 days usually succeeds, becomes less and less frequently successful with increase of the interval and after 90 days usually fails [Ossola (1909), Truffi (1909 and 1910), Tomaszewski (1910), Zinsser, Hopkins and McBurney (1916), Reiter (1924 and 1926), Adachi (1925), Chesney and Kemp (1925), Plaut and Mulzer (quoted by Mulzer and Nothhaas 1926), Nothhaas

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(1927), Stempel and Armuzzi (1927), Manteufel and Worms (1927), Chesney (1930), Frei (1931), Bessemans and de Potter (1933), Gastinel and Pulvenis (1934a)], though some exceptions have been observed by various workers [Truffi (1909), Ossola (1910), Uhlenhuth and Mulzer (1913), Manteufel and Worms (1927), Frei (1931), Orlov (1932), Bessemans and de Potter (1933), Gastinel, Pulvenis and Collart (1936)].

That resistance to superinoculations late in the course of syphilis after a primary testicular or subscrotal infection extends also to other parts of the skin has been shown in sub- and intracutaneous superinoculation experiments by Adachi (1925) ; Chesney and Kemp (1924 and 1925) ; Yaida (1929) ; this author observed a refractory condition also as early as the 50th day) ; Schumacher (1930) ; Rich, Chesney and Turner (1933) ; and Turner (1939). The same refractory condition of the skin to cutaneous and subcutaneous superinoculations and even of the testicular tissue late in the course of syphilis (128-500 days) after a primary infection also of skin other than scrotal was shown by Chesney and Kemp (1924 and 1925) ; Grossmann (1930) ; Schumacher (1930) ; Chesney and Turner (1931) ; Rich, Chesney and Turner (1933) ; but under these conditions positive superinoculations have sometimes occurred when the interval between the first and second inoculation has been only short (28 days, Rich, Chesney and Turner, 1933) or again when it has been longer than 90 days (Schumacher 1930).

The time-factor seems to play also a part between intratesticular and intracutaneous first infections and intravenous superinoculations [Chesney and Turner (1931) ; Misaizu (1933) ; Shime (1934)], as conversely between intravenous first and intracutaneous superinoculations [Chesney and Turner (1931) ; Rich, Chesney and Turner (1933) ; Shime (1935)]. The prevention of positive superinoculations could be observed to depend on the time-interval mostly under these conditions also, but Chesney (1930), Matsumoto (1930) and Pawlow and Kargin (1932) emphasize that generally one should not stress too much the importance of this factor, as the development of immunity is dependent not only on the time interval but also on a number of other factors. According to my experience the immunity persists for the life of infected and untreated rabbits, and Chesney

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(1930) seems to be of the same opinion, stating, after he has dealt with the immunity of syphilis even after intensive treatment, that the resistant state " may continue to be an attribute for life."

In experimental yaws of the rabbit [Ikegami (1926) and Manteufel and Herzberg (1929)] the resistance to superinfection seems to be weaker than in syphilis, and the time-limit for the development of a resistance towards second inoculations appears to be considerably longer, probably more than eight months after appearance of the chancres [Ikegami (1926)].

(II) QUALITY (VIRULENCE) AND QUANTITY OF *S. pallida*

The above facts in experimental syphilis refer only to superinoculations with homologous strains; with heterologous strains the case is different. The result then varies chiefly with the virulence of the strains used in the first and second inoculations respectively, as was first pointed out by Brown and Pearce (1921), who concluded " It is thus clear that in given instances, the resistance acquired as a result of infection with an organism of low virulence may never reach the point of an effectual protection against one of high virulence." On the strength of successful inoculation with heterologous strains carried out at least 120 days after the first inoculation as shown in Table I, Kolle (1926) concluded

TABLE I

Strains used in 1st Inoculation	Strains used in 2nd Inoculation	Number of Rabbits	Positive Results
Truffi . .	Nichols . .	22	12
Nichols . .	Truffi . .	7	4
Truffi . .	Kuznitzky . .	14	4
Nichols . .	Kuznitzky . .	8	3
	Totals .	51	23 = 45.1%

that rabbits acquire only a mono-immunity as contrasted with the pan-immunity acquired by man. That the matter is not so simple as this, may be seen in the review

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by Mulzer and Nothhaas (1926) of experiments by Plaut and Mulzer as follows. They divided the experiments concerned into three groups. In the first cross-inoculations of old strains had been made into animals inoculated at first with "Kolle-Virus" (at that time beyond its 200th passage) and "Mulzer-Virus" which at that time had passed over the 20th passage, the superinoculations being made respectively with "Kolle-Virus" into "Mulzer"-rabbits and "Mulzer-Virus" into "Kolle"-rabbits. No positive result followed cross-inoculations made 4-12 months after the first infection. In the second group were rabbits which had been inoculated either with a strain just transmitted from a human source or with one which had undergone only a few rabbit-passages, *i.e.*, strains which had acquired only a low virulence for rabbits so far as the production of external manifestations was concerned. Superinoculations of these rabbits with "Mulzer" or with "Kolle-Virus" were positive, even 14½ months after the first inoculation. In the third group rabbits first infected with a recent strain did not develop any symptoms if they were superinoculated with a different recent strain as long as 7 to 14½ months later. The results in the second group correspond to those of Brown and Pearce (1921); those of the first and third groups are evidence against Kolle's dictum concerning the "mono-immunity" of syphilitic rabbits. They seem rather to show that the conditions affecting success or otherwise of superinoculation with heterologous strains of a virulence equal to that of the strain used in the first inoculation are similar to the conditions affecting superinoculation with homologous strains. As positive results in Plaut and Mulzer's experiments were not always verified by evidence of spirochætes in the lesions, Mulzer and Nothhaas did not stress the importance of these deductions and undertook further experiments. The results of these experiments as well as those of several other workers are given in Tables II, III, and IV which relate to heterologous superinoculations carried out more than 90 days after the first infection. The superinfection as well as the first inoculation were apparently made by the scrotal or testicular route in all these experiments with the exception of 5 intracutaneous superinoculations carried out by Chesney, Halley and Kemp.

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TABLE II—HETEROLOGOUS CROSS-INOCULATIONS WITH
STRAINS OF APPROXIMATELY EQUAL AGE
(Possible exceptions *, †, and § below)

Names of Authors	Number of Animals	Positive Results
Kolle (1926)	51	23
Nothhaas (1927)	15	2
Manteufel and Worms (1927)	3	2
Chesney, Halley and Kemp (1927)	2	0
Stempel and Armuzzi (1927)	55	12
Mulzer and Nothhaas (1928) *	5	5
Frei (1931) †	22	4
Bessemans and de Potter (1933) §	28	5
Totals	181	53 = 29.3%

* These rabbits were first inoculated with recently isolated strains (1st-3rd passages) and superinoculated with a strain which had undergone 200 subcultures in an artificial medium and had then been transmitted to a rabbit's testis in which it had undergone three passages.

† Eight of these 22 rabbits were first infected with the 2nd-7th passages of strain H and superinoculated with the 7th-12th passages of strain D; as H as well as D were both young strains they are included here.

§ Bessemans and de Potter explain the low figure of positive reinoculations by the belief that, as all their strains were obtained in Ghent at the same period, these strains were more or less biologically related to each other.

Excluding the experiments *, †, and § in this table, 126 rabbits are left, with 39 = 30.9 per cent. positive superinoculations. As Zinsser, Hopkins and McBurney (1916), Reiter (1924) and Orlow (1932, quoted from an abstract) do not state the age of their strains the 223 heterologous superinoculation experiments of these authors with altogether 56 positive results are not considered here.

TABLE III—SUPERINOCULATIONS WITH STRAINS OLDER
THAN THOSE USED IN THE FIRST INOCULATION

Names of Authors, and Strains used in Frei's First Inoculations.	Number of Animals	Positive Results
Adachi (1925)	4	0
Nothhaas (1927)	1	1
Chesney, Halley and Kemp (1927)	9	7
Stempel and Armuzzi (1927)	3	2
Mulzer and Nothhaas (1928)	6	6
Frei: (1927, 1931):—		
Strain D1-D4 *	6	5
Strain D5-D8	3	2
Strain D9-D14 †	6	0
Strain H1-H8 *	8	2
Strain H9-H16†	13	3
Totals	59	28 = 47.5%

* The figure following the letters indicating the strain D and H indicates the number of passages the strain used for the first infection had undergone.

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† For convenience higher passages of strains D and H are included here, all the more as the "ages" of these strains even in the 14th and 16th passages were still considerably lower than that of the old Kuznitzky strain. If, however, these experiments (†) were not included, the totals would be 40 rabbits and 25 = 62.5% positive results.

Note further that, whereas there is a marked difference between the results of the group D₁-D₄ and those of D₉-D₁₄, there is hardly any between groups H₁-H₈ and H₉-H₁₆ in this respect.

TABLE IV—SUPERINOCULATIONS WITH STRAINS YOUNGER THAN THOSE USED IN THE FIRST INOCULATION

Names of Authors, and Strains used in Frei's Superinoculations	Number of Animals	Positive Results
Adachi (1925)	14	5
Stempel and Armuzzi (1927)	20	5
Mulzer and Nothhaas (1928)	5	2
Idem *	7	4
Frei (1927, 1931):—		
Strain D †	13	0
Strain H8	16	1
Strain H ₁₂₋₁₇ †	7	4
Totals	82	21 = 25.6%

* Cfr. note * under Table II.

† Note the difference caused by the higher passages in the superinoculation-experiments with H₁₂₋₁₇ as contrasted with the lower passages H8, but for the same reasons as are given under † below Table III the H₁₂₋₁₇ experiments have been included here. If, however, these experiments † as well as those marked * were not considered here, the totals would be 68 with 19.1% positive results.

‡ D3 in 8 and D6 in 5 rabbits.

From Tables II, III and IV it can be seen that, on the average, the positive results of heterologous superinoculations—carried out later than 90 days after the first infection—between equivalent strains amount to about 29 per cent. if all the experiments in Table II are counted or about 31 per cent. if those marked *, † and § are excluded, that is much lower than Kolle's figure of about 45 per cent. (cfr. Table I). The rate seems to be lower, about 26 per cent. (or about 19 per cent. if the experiments marked * and † in Table IV are excluded), if rabbits first infected with old strains are superinoculated with young strains. On the other hand it is apparently much higher, about 48 per cent. (or about 63 per cent. if the experiments marked † in Table III are not included), when old strains are superinoculated on rabbits first infected with young ones. A conclusion on these points can, however, be derived only from a greater number of

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experiments ; in smaller series there will certainly be exceptions to the general rule respecting the influence of the number of passages on the virulence and the results of cross-inoculation experiments.

But the above respecting the influence of the number of passages seems to apply only to heterologous strains ; with homologous strains, increasing number of passages has hardly seemed to increase the virulence of the strains at all (Frei 1931), when spirochætes of higher passages were used for the superinoculation of animals first infected with lower passages of the same strain. Altogether while I think that age of strain has a definite influence on the results of heterologous superinoculations, these depend also on the speed with which the strains become adapted to the rabbits and possibly also on a biological relationship between the strains ; there will always, therefore, be some individual variations.

That there may be a relation between the quality and quantity of *S. pallida* necessary for a successful testicular infection has been maintained by Zimmermann (1930), van Haelst (1934), Gastinel and Pulvenis (1934*b*) and Thomas and Morgan (1934). On the other hand Chesney and Kemp (1925*a*), as well as Wakerlin (1925) did not see any dependence of the result upon the number of spirochætes in the inoculum, except in regard to the length of the incubation period. All these results refer only to first inoculations ; in these the organism has only to break through the natural defence of the normal rabbit, which apart from the relatively few cases of natural anergy is poor. In reinoculation experiments, on the other hand, the newly inoculated *S. pallida* have to fight against defence barriers built up in response to the first infection, barriers which probably—I dare say certainly—are stronger than the natural resistance of healthy rabbits. In these circumstances differences in the number of *S. pallida* may play quite an important part, and Strempel and Armuzzi (1927*b*), Matsumoto (1930) and Gastinel and Pulvenis (1934*a*) stress the influence of the quantity of the virus on the effects of reinoculation. The difference in the large quantity of the infectious material used for the reinoculation experiments on syphilitic man as compared with that occurring in natural reinoculations may be partly responsible for the successful results in artificial reinoculations.

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III. THE METHODS AND THE SITES OF THE FIRST AND THE SUPERINOCULATION

The work of Adachi (1925), Chesney and Kemp (1924, 1925b), Grossmann (1930), Kato and Iseki (1931), Chesney and Turner (1931), Rich, Chesney and Turner (1933) proved that in rabbits a refractory condition develops towards homologous superinoculations, and it makes no difference whether the first or second inoculations are carried out in the scrotal or the testicular tissue or in the skin elsewhere. Chesney and Turner (1931) and Rich, Chesney and Turner (1933) as well as Shime (1935) showed further that similar relations also exist between first intravenous and second intracutaneous inoculations, and Misaizu (1933) and Shime (1934) between first cutaneous and second intravenous inoculations. The essential condition for the development of an immunity in all these different experiments in which the first infection is not carried out by the testicular or scrotal route is that the skin should take an active part in the manifestations following the first inoculation; the development of the immunity is not at all dependent upon an involvement of the testicular tissue alone.

That refractory conditions also towards heterologous cutaneous or intracutaneous superinoculations are developed by successful primary testicular infections was shown by Adachi (1925), Chesney, Halley and Kemp (1927) and Bessemans and de Potter (1933), but the number of cases is still too small, and further experiments are necessary.

THE QUESTION OF LOCAL IMMUNITY

The site of the first infection has been considered in relation to the possible development of a "local" immunity. The existence of such a local immunity, assumed by Zinsser, Hopkins and McBurney (1916), could not be confirmed by Frei (1931). On the other hand Gastinel, Pulvenis and Nevot (1932) believe that the general immunity starts with the establishment of a local immunity and remains there longest. This view refers to homologous and to some extent also to heterologous superinoculations.

In the hope of elucidating this problem the fate of the inoculum used in these superinoculations has been investi-

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gated. In testicular experiments with homologous strains the implanted tissue particles were absorbed much more quickly on the side which had already been the site of a syphilitic affection than on the opposite one. In later papers Gastinel *et al.* (1934 and 1936) reported that both grafts on either scrotal side, although showing marked difference in absorption, contained virulent spirochætes up to the eighth day after implantation. In their opinion the regional immunity is not dependent upon a spirochetidal property, but is consequent only on a new adaptation of the tissue towards the spirochætes. Contrary to some part of these observations Strempel (1928) found that, in rabbits superinoculated with homologous spirochætes after the 90th day, the tissue particles constituting the inoculum were found on removal to be infective for new rabbits up to the 25th day. In histological examinations, the homologous spirochætes showed at first more evidence of degeneration in the periphery than in the central parts of the tissue used as inoculum, and Strempel and Armuzzi (1927*a*) attribute this to the effect of spirochetolysins.

In this connection I would refer to Kolle and Schlossberger (1926) who, by lymph-gland-transfers and cross-inoculations, came to the conclusion that in heterologous superinoculations in which no external effect of the superinoculation was to be seen the spirochætes of the superinoculation entered the popliteal lymph glands. Also to Strempel and Armuzzi (1927*b*) and Armuzzi (1928*b*) who believed they had proved that $2\frac{1}{2}$ months after a negative heterologous superinoculation the remnants of the inoculum still contained the superinoculated spirochætes. Armuzzi (1928*a*) claimed to have proved by cross-inoculations that in the case of a heterologous superinoculation-chancres of a rabbit, which 28 days previously had been unsuccessfully superinoculated on the same area with the homologous strain, the spirochætes of both these strains were to be met with. In the same paper Armuzzi emphasized as important characteristics of heterologous superinoculation chancres, *inter alia*, the longer incubation time, the limited development, the almost complete absence of the "myxomatous substance" and the quick regression. These special features must certainly be due to the effect of the rabbit's defensive agencies on the spirochætes.

IV. THE DEPENDENCE OF RESISTANCE TO SUPERINOCULATION ON THE REACTION OF THE RABBIT TOWARDS THE FIRST INOCULATION

The effect of the reaction to the first inoculation, *i.e.*, the development and the degree of syphilitic manifestations, in building a resistance to superinoculation, has been emphasized by Reiter (1924), Adachi (1925), Strempel and Armuzzi (1926), Brandt (1930, 1934), Brown (1930), Chesney (1930), Grossmann (1930), Pawlow and Kargin (1932), and Gastinel and Pulvenis (1934*a*), amongst whom I may quote Brown as follows: "protection against a second infection is merely an expression of a reaction to a first infection—that the results of reinoculation are determined by the progress and efficiency of this reaction——."

It is therefore understandable that rabbits which show no sign after a primary infection can also be successfully superinoculated late in the course of the disease. Thus Manteufel and Richter (1926), Bessemans and Vlaeyan (1928), Kigasawa (1929), Prigge and Rothermundt (1927), and Grossmann (1930) successfully superinoculated with homologous strains rabbits which had not developed syphilitic manifestations after the first intravenous, subscrotal, or subcutaneous inoculations but whose infection was proved by positive lymph-gland-transfers in the cases of Prigge and Rothermundt and Kigasawa. The time interval in Manteufel and Richter's experiments was only 86 days, but in those of the other workers it was more than 90 days. According to Kigasawa and to Grossmann, it seems possible, however, that a resistance to superinoculation may develop even in animals which do not show any clinical sign after the first infection, if the latter persists for a long time and the second inoculation is carried out after a rather prolonged interval. A confirmation of this view even towards a heterologous strain (consider note § under Table II) may be seen in a rabbit (Bessemans and de Potter (1933) the first infection of which was checked by a positive lymph-gland-transfer. On the other hand several cases were reported by Bessemans and de Potter (1933) which might be exceptions to the view expressed in this paragraph, but the experiments of these workers were checked by lymph-gland-transfer in only a few of the animals. In one such case

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a rabbit which was first infected without external signs (intratesticularly, lymph-gland-transfer positive) resisted an homologous intratesticular reinoculation even 63 days after the first infection.

That apparently also repeated homologous superinoculation without a local response may effect the result of a subsequent heterologous superinoculation may be seen from experiments of Manteufel and Worms (1927), which are summarized in Table V.

TABLE V—HOMOLOGOUS AND HETEROLOGOUS SUPERINOCULATION OF RABBITS IN THE LATENT STAGE OF THE INFECTION AFTER SPONTANEOUS HEALING OF THE EXTERNAL SIGNS DUE TO THE FIRST INFECTION

Rabbit Number	Strain and Result of 1st Inoculation.	Strain and Result of 2nd Inoculation	Strain and Result of 3rd Inoculation	Strain and Result of 4th Inoculation
2,312	Nichols pos.	Nichols neg.	Nichols neg.	R.G.A. neg.
2,193	Nichols pos.	Nichols neg.	Nichols neg.	R.G.A. neg.
2,673	Nichols pos.	Nichols neg.	Nichols neg.	R.G.A. neg.
2,215	Nichols pos.	Nichols neg.	Nichols neg.	R.G.A. neg.
418	Nichols pos.	R.G.A.* pos.		
419	Nichols pos.	R.G.A. pos.		
420	Nichols pos.	R.G.A. neg.		

* R.G.A. = Reichsgesundheitsamts-strain.

As the table shows, these authors superinoculated four Nichols rabbits after spontaneous healing in the latent stage (more than three and four months after the 90th day-limit) twice with the same strain (at intervals ranging from more than three to four months) and no reinoculation chancre developed. A third superinoculation was made with a heterologous strain (after a further interval of more than six, or in two cases more than seven months) and even then no manifestation appeared; in three other Nichols-rabbits, however, the heterologous superinoculations (carried out about seven months after the first inoculation)—without the two preceding homologous reinoculations—led in two cases to reinoculation-chancres. In the first four rabbits it was probably the repeated inoculation of *S. pallida* which increased—in an immunological respect—the effect of the first inoculation.

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In order to observe the effect of a maximum response to the first inoculation on subsequent reaction to super-inoculation and to see if there is really only a "mono-immunity" in syphilitic rabbits I carried out the following experiments.

Contrary to previous practice in which rabbits had been mostly infected with one strain and exceptionally only from rabbits inoculated with two strains or with spirochaetes of two different strains on each side of the scrotum (Armuzzi, 1928a), I inoculated 27 rabbits by the testicular route with a mixture (tissue emulsion) of three different, equivalent, old strains of *S. pallida* taken from three different primary sores. Sixteen of these animals were unilaterally inoculated with a mixture of *S. pallida* obtained from chancres of a Nichols-, a R.G.A.- and a Kuznitzky-strain rabbit, and 11 were infected in both testes with a mixture of chancres excised from a Nichols-, a R.G.A.- and a Truffi-strain rabbit respectively.

The outstanding features of these infections were : (1) a marked shortening of the incubation period in about half (14) of the animals ; (2) the occurrence of extraordinary large (potato-size) chancres or orchitic manifestations in 12 animals and of very large-sized ones (walnut) in six ; (3) the relatively frequent development of metastatic chancres in the second testis in 11 of the 16 rabbits of the first group ; (4) the long duration of the clinical (primary testicular or scrotal) symptoms in 10 out of 17 rabbits (in 5 of these 10 rabbits 5 to 7 months and in another even 10 months !). All these observations seem to be evidence of a special virulence of the mixture of the three strains of *S. pallida*. As all these 10 rabbits had syphilitic manifestations of extraordinary size (7) or very large size (3) I agree with Uhlenhuth and Grossmann (1926) who could not confirm Brown and Pearce's "law of the inverse proportions" in so far as the largest manifestations were those which persisted also for the longest time.

Ten animals (five of the first group, four of the second series and a fifth representing the first passage of one rabbit out of the second group and in the same way showing also a strong reaction after the first inoculation) were available for heterologous superinoculation experiments, when they had become free of all external signs. Five animals of the first group (unilaterally infected

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with the Nichols, R.G.A. and Kuznitzky strains), four of which had developed a metastatic lesion on the second testis, were superinoculated into both testes with the Truffi strain 431 days after the first inoculation. None of these developed any manifestation during 155 days in one and 414 days in the other cases. Six controls infected with the Truffi strain all showed typical primary affections. Four rabbits of the second group (inoculated into both testes with the Nichols, R.G.A. and Truffi strains) and a fifth rabbit inoculated with the first passage of this mixture were intratesticularly superinoculated with the Kuznitzky strain, in two cases 286 and 386 days and in the three others, 265 days after the first inoculation. Contrary to 5 of the 6 controls which got primary sores or orchitis, none of them developed any clinical manifestation at all in an observation period of 153 days.

Thus all these 10 animals, 9 of which had first been simultaneously infected with a mixture of three old strains and the 10th with the first passage of such a mixture, were refractory to a heterologous superinoculation with a fourth old strain carried out long after the three-months-limit of Kolle. All of them therefore developed a pan-immunity—which according to Kolle is obtainable only in about half the heterologous superinoculations in rabbits. The experiments reported above seem to show that the untreated rabbit which reacts very strongly to the first inoculation—carried out simultaneously with three old strains—is able to develop not merely a mono-immunity but a non-strain-specific or pan-immunity.

Further experiments (I have not had the opportunity of carrying out further experiments myself since 1933) on a larger scale are of course necessary to repeat and vary the investigation, especially to see also if a simultaneous first infection with a mixture of young strains is also able to bestow upon rabbits a pan-immunity against superinoculations with old strains, and further if under these and the above conditions a pan-immunity can be obtained also in rabbits treated late in the course of the first infection (cfr. Chesney, Halley and Kemp, 1927, and Chesney, Turner and Grauer, 1933).

Apart from these questions, it seems worth while to examine the sera of rabbits which have been inoculated

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simultaneously with several old strains for the presence of protective antibodies. I myself could not find such antibodies when I examined a pooled serum of five rabbits which had been inoculated with three old strains and reinoculated with a fourth old strain; these experiments using a mixture of equal amounts of immune sera and suspensions of *S. pallida* (of one of the strains used for the first inoculation) were, however, much too small and they were carried out 9½ years ago in the way that was then customary. Since the parabiosis experiments of Krantz (1933) and of Tani and Aikawa (1936) and also the work of Tani, Saito and Funada (1935), of Tani and Ogiuti (1936) and especially of Turner (1939) using mixtures of serial dilutions of spirochetal emulsions and immune sera or (Turner) mixtures of the "minimal chancre doses" of a spirochetal emulsion and immune sera have proved the definite existence of a certain though small amount of such protective antibodies, it is probable that sera of rabbits inoculated simultaneously with several strains of spirochaetes might show also a greater amount of protective antibodies and thus give further support for the importance of humoral antibodies—in addition to that of the fixed tissue cells—in the acquired immunity to syphilis.

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REFERENCES

- ADACHI, Y. (1925) *Acta derm., Kyoto*, **5**, 42.
ARMUZZI, G. (1928 a) *Derm. Z.*, **53**, 750; (1928 b) *G. Ital. Derm. Sif.*, **69**, 381.
BESSEMAN, A., and DE POTTER, Fr. (1933) *Arch. int. Méd. exp.*, **8**, 47.
BESSEMAN, A., and VLAEYEN, N. (1929) *Arch. int. Méd. exp.*, **4**, 471.
BRANDT, R. (1930) *VIII. Int. derm. Congr. Copenhagen*, 1030; (1934) *Derm. Z.*, **69**, 193.
BREINL, F. (1935) *Z. Immun. Forsch.*, **84**, 195.
BREINL, F., and WAGNER, R. (1929) *Z. Immun. Forsch.*, **60**, 23.
BROWN, W. H. (1930) *VIII. Int. derm. Congr. Copenhagen*, 220.
BROWN, W. H., and PEARCE, L. (1921) *Proc. Exp. Biol. and Med.*, **18**, 255.
CHESNEY, A. M. (1926) *Medicine, Baltimore*, **5**, 463; (1930) *Amer. J. Syph.*, **14**, 289.
CHESNEY, A. M., HALLEY, CH. R. L., and KEMP, J. E. (1927) *J. Exp. Med.*, **46**, 223.
CHESNEY, A. M., and KEMP, J. E. (1924) *J. Exp. Med.*, **39**, 553; (1925 a), *ibid.* **41**, 479; (b) *ibid.* **42**, 17.

EXPERIMENTAL RABBIT-SYPHILIS

- CHESNEY, A. M., and TURNER, B. T. (1931) *Johns Hopk. Hosp. Bull.*, **48**, 90.
- CHESNEY, A. M., TURNER, B. T., and GRAUER, FR. H. (1932) *Trans. Ass. Amer. Phys.*, **47**, 182; (1933) *Johns Hopk. Hosp. Bull.*, **52**, 145.
- FREI, W. (1927) *Dtsch. med. Wschr.*, **53**, 1174; (1931) *ibid.* **57**, 973.
- GASTINEL, P., DELARUE, I., NÉVOT, A., and COLLART, P. (1934) *Bull. Soc. franç. Derm. Syph.*, **41**, 1679.
- GASTINEL, P., and PULVENIS, R. (1934a) *Bull. méd.*, **48**, 253; (b) *Bull. Soc. franç. Derm. Syph.*, **41**, 330.
- GASTINEL, P., PULVENIS, R., and COLLART, P. (1936) *Bull. Soc. franç. Derm. Syph.*, **43**, 1141 and 1145.
- GASTINEL, P., PULVENIS, R., and NÉVOT, A. (1932) *Bull. Soc. franç. Derm. Syph.*, **39**, 1382.
- GROSSMAN, H. (1930) *Arch. Hyg. Berl.* **103**, **49**; (1933) *Z. Immun. Forsch.*, **79**, 495.
- IKEGAMI, Y. (1926) *Acta. Derm., Kyoto*, **8** (Abstr. Sect.) 816.
- KATO, T., and ISEKI, K. (1931) *Lues*, **6**, 8.
- KIGASAWA, T. (1929) *Z. Immun. Forsch.*, **60**, 69.
- KOLLE, W. (1922) *Dtsch. med. Wschr.*, **48**, 1301; (1924) *ibid.* **50**, 1235; (1926) *ibid.* **52**, 11.
- KOLLE, W., and SCHLOSSBERGER, H. (1926) *Dtsch. med. Wschr.*, **52**, 1245.
- KRANTZ, W. (1933) *Arch. Derm. Syph.*, **168**, 109.
- MANTEUFEL, P., and HERZBERG, K. (1929) *Med. Welt*, **3**, 297; (1933) *Z. Immun. Forsch.*, **79**, 482.
- MANTEUFEL, P., and RICHTER, A. (1926) *Dtsch. med. Wschr.*, **52**, 2113.
- MANTEUFEL, P., and WORMS, W. (1927) *Zbl. Bakt. I. Orig.*, **102**, 23.
- MATSUMOTO, SH. I., (1930) *VIII. Int. Congr. Derm. Copenhagen*, 254; (1938) *Acta Derm., Kyoto*, **31**, 51.
- MISAIZU, H. (1933) *Acta Derm. Kyoto*, **21**, 72.
- MULZER, P., and NOTHHAAS, R. (1926) *Arb. Reichsgesundh. Amt*, **57**, 155; (1928) *Münch. med. Wschr.*, **75**, 169.
- NOTHHAAS, R. (1927) *Dtsch. med. Wschr.*, **53**, 102.
- ORLOV, S. (1932) *Trudy 3 vses. S-ezda Bořba vener. Bol.* 245 and 254; *Abstract Zbl. Haut u. Geschlkr.* (1934) **47**, 85.
- OSSOLA, S. (1909) *Giorn. ital. Mal. vener.*, **50**, 171; (1910) *ibid.* **51**, 130.
- PAWLOW, S. T., and KARGIN, W. A. (1932) *Derm. Z.*, **64**, 316.
- PRIGGE, R., and ROTHERMUNDT, M. (1927) *Derm. Z.*, **50**, 169.
- REITER, H. (1924) *Zbl. Bakt. I. Orig.*, **92**, 534; (1926) *Klin. Wschr.*, **5**, 1356.
- RICH, A. R., CHESNEY, A. M., and TURNER, B. T. (1933) *Johns Hopk. Hosp. Bull.*, **52**, 179.
- SCHLOSSBERGER, H., and WORMS, W. (1932) *Arch. Derm. Syph.*, **164**, 628; (1932) *Med. Klinik*, **28**, 1003.
- SCHUMACHER, C. (1930) *VIII. Int. derm. Congr. Copenhagen*, 988.
- SHIME, K. (1934) *Lues*, **11** (Abstr. Sect.) **15**; (1935) *ibid.* **12** (Abstr. Sect.) **11**.
- STREMPER, R. (1928) *Derm. Z.*, **53**, 615.
- STREMPER, R., and ARMUZZI, G. (1926) *Derm. Z.*, **48**, 129; (1927) (a) *Derm. Z.* **50**, 423; (b) *Dtsch. med. Wschr.*, **53**, 1134.

BRITISH JOURNAL OF VENEREAL DISEASES

- TANI, T., and AIKAWA, S. (1936) *Jap. J. exp. Med.*, **14**, 465; (1940) *ibid.* **18**, 39.
- TANI, T., SAITO, K., and FUNADA, H. (1935) *Zbl. Bakt. I. Orig.*, **134**, 232.
- TANI, T., and ÔGIUTI, K. (1936) *Jap. J. exp. Med.*, **14**, 457.
- THOMAS, CL. S., and MORGAN, H. J. (1934) *J. exp. Med.*, **59**, 297.
- TOMASCZEWSKI, E. (1910) *Berl. klin. Wschr.*, **47**, 1447.
- TRUFFI, M. (1909) *Zbl. Bakt. I. Orig.*, **52**, 555; (1910) *ibid.* **54**, 337.
- TURNER, T. B. (1939) *J. exp. Med.*, **69**, 867; (1939) 3rd Intern. Congr. Microbiology, New York, 636.
- UHLENHUTH* P., and GROSSMAN, H. (1926) *Arch. Derm. Syph.*, **152**, 708; (1927) *Zbl. Bakt. I. Orig.*, **104**, 166; (1928) *Z. Immun. Forsch.*, **55**, 380.
- UHLENHUTH, P., and MULZER, P. (1913) *Arb. ReichsgesundhAmt*, **44**, 307.
- VAN HAELST, J. (1934) *Derm. Z.*, **69**, 212.
- V. VÁSÁRHELYI, J. (1936) *Z. Immun. Forsch.*, **89**, 296.
- WAKERLIN, G. E. (1926) *J. infect. Dis.*, **38**, 323.
- YAJIDA, H. (1929) *Jap. med. World*, **9**, 183.
- ZIMMERMANN, E. (1930) *Arch. Hyg. Berl.*, **103**, 269.
- ZINSSER, H., HOPKINS, J. G., and MCBURNEY, M. (1916) *J. exp. Med.*, **24**, 561.

IV

THE LORENZ FLOCCULATION TEST FOR THE DIAGNOSIS OF SYPHILIS IN BLOOD AND CEREBROSPINAL FLUID

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Lorenz (1940) described a rapid flocculation test for syphilis adapted for cerebrospinal fluid, using a modified Laughlen antigen. He obtained very satisfactory results when compared with a complement fixation procedure, and the test was much quicker to perform. It would therefore be valuable as an easy and rapid method of examination in some laboratories, provided it gave no false positive reaction and yielded a reasonably high proportion of correct positive results, as compared with the Wassermann.

This paper describes the results of Lorenz tests of 510 cerebrospinal fluids, and the application of a modification of the test to blood.